homologs, and wherein all but one of said *sap*A homologs are altered by inserting DNA cassettes encoding heterologous proteins into the coding sequences of said *sap*A homologs.

REMARKS

Amendment

Attached hereto is a marked-up version of the changes made to the specification and claims by the current amendment. The attached page is captioned "Version with markings to show changes made."

The 35 U.S.C. §112 Rejection

Claims 1, 5-9, 18 and 10-13, 15-17 were rejected under 35 U.S.C. §112, first paragraph, for lack of deposit. The rejection is respectfully traversed.

Claim 1 is drawn to a genetically engineered mutant *C. fetus* strain containing a DNA cassette inserted into the coding sequence of a *sap*A homolog. The expression of said DNA cassette results in surface expression of a chimeric protein comprising a heterologous protein encoded by the DNA cassette. The instant

specification teaches mutant *C. fetus* strain was derived from wild type *C. fetus* strain 23D into which a DNA cassette was inserted using plasmids pKO500 or pKO505. *C. fetus* strain 23D has been deposited at ATCC as PTA-4754, *E. coli* HB101 with plasmid pK0500 was deposited as PTA-4751, and *E. coli* DH5-α with plasmid pK0505 was deposited as PTA-4752.

Consequently, Applicants submit that one of ordinary skill in the art would readily derive a mutant *C. fetus* strain as claimed in claim 1 using the materials and methods disclosed herein. Similarly, mutant *C. fetus* strain containing insertion of DNA cassettes in multiple *sapA* homologs as claimed in claim 18 can be readily obtained using the deposited materials.

The Examiner contends that reciting *sapA* homologs would read on *sapA1*, *sapA2*, *sapB*, *sapC*, *sapD*, *sapE* and *sapF*. Applicants respectfully disagree. Applicants submit that it is clear to one of ordinary skill in the art that *sapB*, *sapC*, *sapD*, *sapE* and *sapF* are not *sapA* homologs. These other genes are different and distinct from *sapA* homologs, and they serve functions different from that of *sapA* homologs.

Claim 10 is drawn to a mutant *C. fetus* strain expressing a *recA* mutation. Applicants submit that an example of such *C. fetus* strain has been deposited at ATCC as PTA-4753.

Claim 15 is drawn to a strain of *Escherichia coli.* modified to express the surface array proteins C, D, E and F of *C. fetus*. *E. coli* DH5-α with plasmid pIR100 that encodes sapCDEF has been deposited at ATCC as PTA-4750. Therefore, one of ordinary skill in the art can readily use the plasmid to construct the claimed bacterial strain.

In view of the above deposits, Applicants respectfully request that the rejection of claims 1, 5-9, 18 and 10-13, 15-17 under 35 U.S.C. §112, first paragraph, be withdrawn.

The 35 U.S.C. §102 Rejection

Claims 1, 6-8 were rejected under 35 U.S.C. §102(a) as being anticipated by **Dworkin** et al. (March 1996). The rejection is respectfully traversed.

First of all, Applicants briefly summarize the present invention as follows. *Campylobacter fetus*, a bacterial pathogen of ungulates and humans, is covered by a paracrystalline surface (S-) layer composed of high molecular weight S-layer proteins (SLP) that

masks most of the underlying gram-negative surface features. The S-layer renders *C. fetus* cells resistant to serum killing by prohibiting the binding of C3b, and the S-layer proteins themselves may change, permitting antigenic variation (instant specification, page 2).

Campylobacter fetus accomplishes this antigenic variation by posssessing 7-9 highly homologous gene cassettes, called *sapA* homologs (*sapA*, *sapA1*, *sapA2*, etc.) each of which encode a different S-layer protein. Each of these homologs contains a 5' region of about 600 base pairs which is completely conserved from homolog to homolog and is necessary for binding of the S-layer protein encoded by that homolog to the lipopolysaccharide molecule anchored in the bacterial outer membrane. The remainder of the open reading frame (ORF) is different for each homolog but semi-conserved regions exist. Wild type *C. fetus* strains are able to rearrange their chromosomal DNA so that the *sapA* homolog positioned downstream of a unique promoter is then expressed (Applicants' specification, pages 2-3).

The mechanism of DNA rearrangement in *C. fetus* was shown in Figure 3. There are multiple *sapA* homologs but only one promoter that can drive the expression of one *sapA* homolog. Hence,

there is only one *sapA* homolog expression at any time. Through DNA inversion mediated through the 5' conserved regions of any two *sapA* homologs as shown in Figure 3, the promoter is moved from one *sapA* homolog to another *sapA* homolog, resulting in the expression of a different *sapA* homolog on the cell surface.

The generation and genotypes of mutant *C. fetus* of the present invention were shown in Figures 1A and 2D. Focusing on Figure 1A, under the column of predicted genotypes, the first genotype shows a wild type genotype in which the promoter is driving the expression of one *sapA* homolog. Consequently, surface expression of *sapA* renders the wild type *C. fetus* cells resistant to serum killing (denoted as S+ under the column of phenotypic resistance). The second genotype shows the insertion of a DNA cassette encoding chloramphenicol resistance into the coding region of *sapA*. The insertion disrupts the expression of *sapA*. Consequently, there is no surface expression of *sapA* and the cells are sensitive to serum killing (S-). However, the promoter is driving the expression of the inserted DNA and thus the cells are chloramphenicol resistant (C+).

Majority of these serum sensitive chloramphenicol resistant *C. fetus* mutants are killed by serum. However, some survivors were isolated upon selection in serum. These survivors are revertants in which the promoter that used to drive the expression of the chloramphenicol resistant insert was translocated to another *sapA* homolog (the third genotype in Figure 1A). Surface *sapA* homolog expression is restored but the cells no longer express the chloramphenicol resistant insert. Consequently, the cells are serum resistant and chloramphenicol sensitive.

These serum resistant chloramphenicol sensitive *C. fetus* cells can further be mutated by inserting a DNA cassette encoding kanamycin resistance into the coding region of *sapA2* (the fourth genotype in Figure 1A). The coding sequences for *sapA* and *sapA2* in these cells are disrupted by the DNA inserts and there is no surface expression of *sapA* homolog. Therefore, the cells are serum sensitive. However, upon selection in serum, revertants were isolated in which the promoter was translocated to a *sapA* homolog other than the disrupted *sapA* or *sapA2* (the last two genotypes in Figure 1A). Surface *sapA* homolog expression is restored and the cells are serum resistant.

Claim 1 is drawn to a genetically engineered mutant *C. fetus* strain containing a DNA cassette inserted into the coding sequence of a *sap*A homolog. The expression of said DNA cassette results in surface expression of a chimeric protein comprising a heterologous protein encoded by the DNA cassette. In contrast, **Dworkin** et al. did not teach or suggest a mutant *C. fetus* as claimed herein.

The Examiner contends in paragraph 18 that "the reference does disclose, teach, suggest the insertion of a DNA cassette encoding a foreign heterologous protein results in the expression of an altered sapA homolog being surface expressed". Applicants respectfully disagree.

Dworkin et al. only teach loss of cell surface S-layer expression due to insertion of a foreign heterologous protein kanamycin (Figure 1, K100 and K300 series; Figure 2) because "insertion of km at base pair 127 of the SLP gene cassette ORF was known to interrupt SLP transcription" (page 1242, right column, lines 21-22). Therefore, inserting the kanamycin DNA cassette into the coding region of *sapA2* disrupts the translation of *sapA2* and no *sapA2* protein is expressed on the cell surface. Consequently, the

cells become serum sensitive (Figure 6, second line). Only after DNA inversion that places the promoter upstream of an unaltered *sapA* homolog does the cells regain surface expression of an unaltered *sapA* and becomes serum resistant (Figure 6, third line). Hence, **Dworkin** et al. indeed teach away from the present invention in that it teach loss of cell surface S-layer expression due to insertion of a foreign heterologous protein, whereas claim 1 of the instant invention is drawn to a mutant *Campylobacter fetus* that expresses a foreign heterologous protein on the surface.

Claim 6 is drawn to a mutant strain of *Campylobacter fetus* that contains a DNA cassette comprising a heterologous protein inserted between the 5' binding region and the 3' secretion signal region of a *sapA* homolog. Claim 7 is drawn to a strain of *Campylobacter fetus* that contains a DNA cassette encoding a heterologous protein, wherein said DNA cassette has the 3' secretion signal region but not the 5' binding region of a *sapA* homolog. **Dworkin** et al. did not teach or suggest a DNA cassette encoding a heterologous protein flanked by the 3' secretion signal region of a *sapA* homolog with or without the 5' binding region of a *sapA* homolog.

Claim 8 is drawn to a mutant *Campylobacter fetus* strain of claim 1 that expresses on the cell surface a heterologous protein such as an antigen or a therapeutic agent. As discussed above, **Dworkin** et al. did not teach or suggest a mutant strain that expresses foreign heterologous protein on the cell surface as claimed herein. Since the identical invention must be shown in as complete detail as is contained in the instant claims, **Dworkin** et al. do not anticipate claims 1, 6-8 of the present invention. Accordingly, Applicants respectfully request that the rejection of claims 1, 6-8 under 35 U.S.C. §102(a) be withdrawn.

Claims 1, 6-8 were rejected under 35 U.S.C. §102(b) as being anticipated by **Blaser** et al. (November 1994). The rejection is respectfully traversed.

The Examiner asserts in paragraph 24 that the reference "discloses that a truncated 50 kDa S-layer protein was surface expressed, although it was in minimal amounts". Applicants respectfully disagree.

Applicants reiterate that inserting a DNA cassette encoding kanamycin resistance into the coding region of a *sapA* homolog gene as shown in **Blaser** et al. disrupts the translation of the

sapA homolog and the truncated *sapA* homolog is not transported to the cell surface (see abstract in **Blaser** et al.). To determine whether truncated S-layer proteins were transported to the cell surface, the cell surface S-layer proteins were extracted by water and the presence or absence of the truncated protein was examined by western blot (page 455, column 2, second paragraph). As shown in Figure 4, the truncated 50 kDa S-layer protein was not detected in the water-extracted fractions. Rather, the truncated protein was associated with the cellular fraction (Figure 4, lane P). These results indicate that truncated S-layer proteins were not transported to the cell surface.

To resolve the issue of apparent minimal expression of truncated S-layer proteins, **Blaser** et al. examined the mutant bacteria under freeze-etch electron microscopy (page 456, column 1, second paragraph). It was found that the characteristic paracrystalline S-layer structure was displayed on only 2% of the cells, and the conclusion is that within the mutant population there are small number of cells in which a switch to expression and export of a full-length (not truncated) S-layer protein had occurred (page 456, column 1, last sentence). In other words, the truncated S-layer protein resulted from DNA insertion is not expressed on the cell

surface; however, a small number of mutant cells become revertants in which the promoter is translocated from the altered *sapA* homolog to an unaltered wild type *sapA* homolog. Subsequently, wild type (unaltered) surface *sapA* homolog expression is restored and the cells become serum resistant (abstract; page 459, column 1, last paragraph).

The Examiner further contends that "in addition to the expression of the altered sapA homolog on the surface, expression of additional sapA coding sequences was found based upon the appearance of additional *sapA* protein bands on a immunoblot gel". Applicants respectfully disagree.

First of all, as described above, there are multiple *sapA* homolog but there is only one promoter for *sapA* expression. Therefore, each cell can only express one *sapA* homolog on the cell surface. The detection of multiple (or additional) *sapA* proteins does not indicate expression of multiple *sapA* homologs on the cell surface. Rather, these results indicate that in a population of mutant cells, different cells are expressing different full-length *sapA* homologs. These full-length *sapA* homologs are expressed on cells that are revertants (not the original mutants). Secondly, immunoblot on

whole cell lysates as shown in **Blaser** et al. does not indicate the proteins are expressed on cell surface. Immunoblot on whole cell lysates only detects the presence or absence of the proteins in the cells. It does not determine where the proteins are expressed in the cells. Other methods (such as the water extraction method described above) are required to show surface expression of the *sapA* protein.

In summary, **Blaser** et al. teach insertion of a DNA cassette encoding a foreign heterologous protein into the coding sequence of a *sapA* homolog results in the loss of cell surface expression of that *sapA* homolog. **Blaser** et al. did not teach or suggest a genetically engineered mutant *C. fetus* strain containing a DNA cassette inserted into the coding sequence of a sapA homolog, wherein the expression of said DNA cassette results in surface expression of a chimeric protein comprising a heterologous protein encoded by the DNA cassette. Since **Blaser** et al. did not teach or suggest each and every aspect of the present invention, **Blaser** et al. do not anticipate the instant invention. Accordingly, Applicants respectfully request that the rejection of claims 1, 6-8 under 35 U.S.C. §102(b) be withdrawn.

The 35 U.S.C. §112 Rejection

Claims 1, 5-13, 15-18 were rejected under 35 U.S.C. §112, first paragraph, for lack of written description. The rejection is respectfully traversed.

In view of the deposits of bacteria and plasmids described above, Applicants submit that the written description requirement has been satisfied in such a way as to reasonably convey to one skilled in the art that the inventors had possession of the claimed invention at the time the application was filed. Accordingly, Applicants respectfully request that the rejection of claims 1, 5-13, 15-18 under 35 U.S.C. §112, first paragraph, be withdrawn.

Claim 11 was rejected under 35 U.S.C. §112, second paragraph, as being indefinite. The rejection is respectfully traversed.

Claim 11 has been amended to recite a strain containing a DNA cassette inserted into the coding sequence of a *sap*A homolog, and expression of said DNA cassette results in surface expression of a chimeric protein comprising said heterologous protein. Applicants submit that the claim has been amended to answer the questions raised in the Office Action and clearly define the components of the

chimeric protein. Accordingly, Applicants respectfully request that the rejections of claim 11 under 35 U.S.C. §112, second paragraph, be withdrawn.

This is intended to be a complete response to the Final Office Action mailed July 18, 2002. If any issues remain outstanding, the Examiner is respectfully requested to telephone the undersigned attorney of record for immediate resolution.

Respectfully submitted,

Date: Dec 31, 2002

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VERSION WITH MARKINGS TO SHOW CHANGES MADE

IN THE CLAIMS:

Claim 1 has been amended as follows:

1. (amended four times) A genetically engineered mutant *C. fetus* strain containing a DNA cassette inserted into the coding sequence of a *sap*A homolog of said strain, wherein said ~~expressing one or more altered *sap*A homologs on cell surface, each of said altered *sap*A homolog is altered by inserting a~~ DNA cassette ~~that~~ encodes a heterologous protein ~~into the coding sequence of said *sap*A homolog~~ and expression of said DNA cassette results in surface expression of a chimeric protein comprising said heterologous protein.

Claim 11 has been amended as follows:

11. (twice amended) The mutant *C. fetus* strain of claim 10, wherein said strain contains a ~~chimeric protein comprising a heterologous antigen and a *sap*A homolog~~ DNA cassette inserted into the coding sequence of a *sap*A homolog of said strain, and expression of said DNA cassette results in surface expression of a chimeric protein comprising said heterologous protein.

Claim 18 has been amended as follows:

18. (thrice amended) The mutant *C. fetus* strain of claim 1, wherein said mutant strain contains from about 7 to about 9 *sap*A homologs, and wherein all but one of said *sap*A homologs are altered by inserting DNA cassettes encoding heterologous proteins into the coding sequences of said *sap*A homologs ~~comprises more than one altered *sap*A homolog and only one unaltered *sap*A homolog~~.